Investigating Genetic Control of the Tumor Microenvironment by Spatial Functional Genomics

Mount Sinai

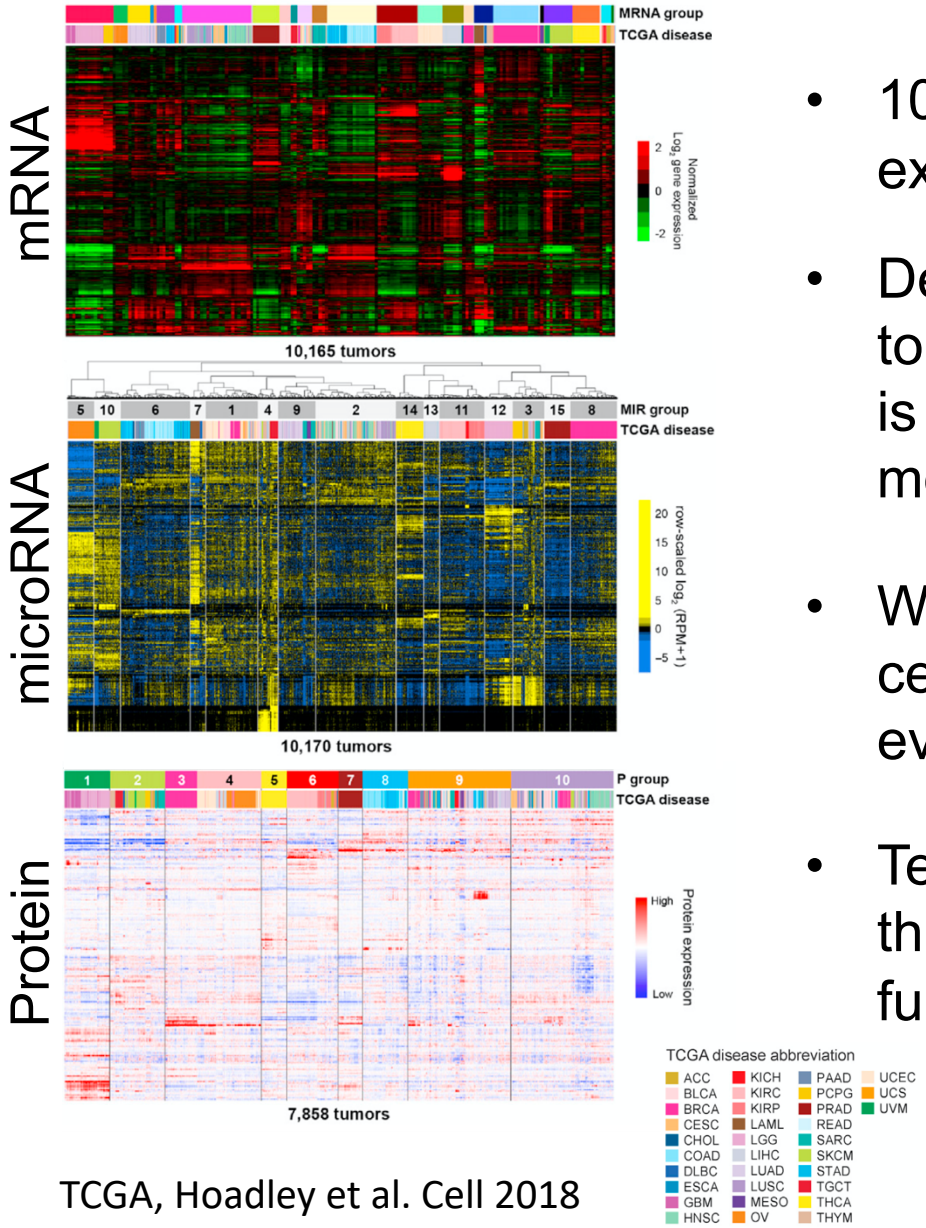
Brian Brown, PhD

Precision Immunology Institute

Genetics and Genomic Sciences

Icahn School of Medicine at Mount Sinai

Major changes in gene expression underlie tumor biology
Challenge: Identifying essential genes in tumor fitness



- 1000s of genes differentially expressed in tumors
- Determining each genes contribution to different aspects of tumor biology is one of the major goals of the modern era of cancer biology
- Which genes are used by cancer cells to alter their phenotype and evade the immune system.
- Technologies needed for high-throughput gene perturbation and functional analysis.

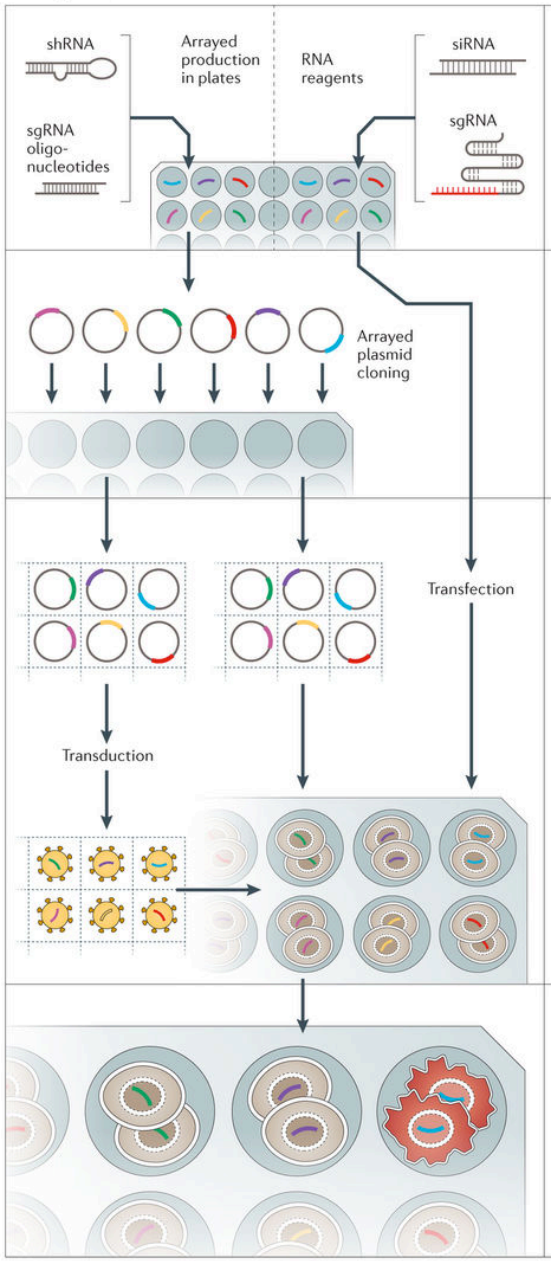
TCGA, Hoadley et al. Cell 2018

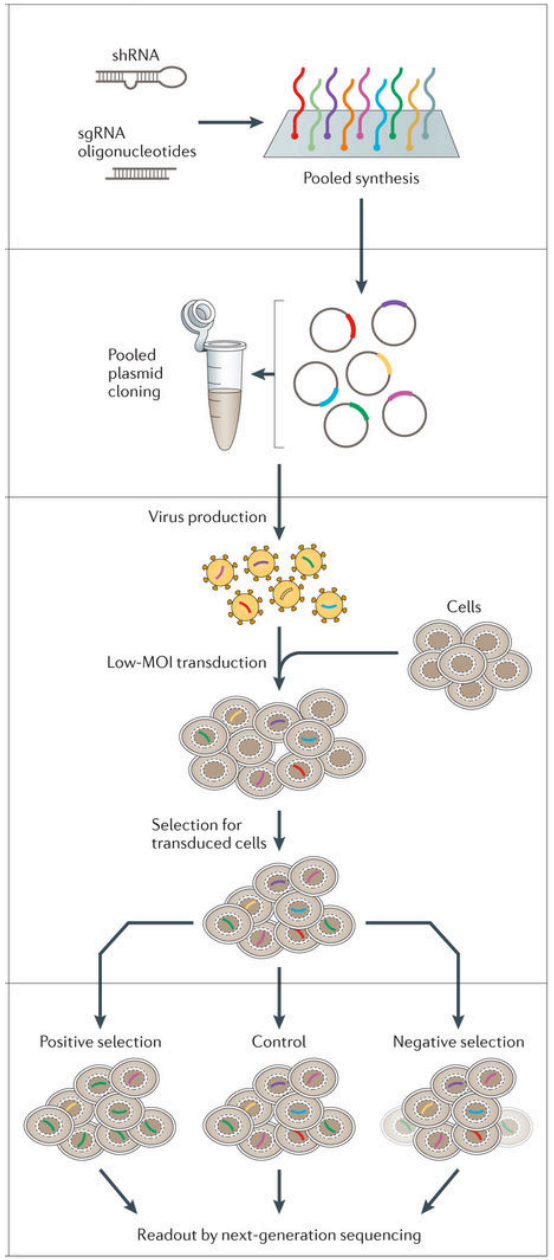
Reagent Synthesis

Library Construction

Cell Targeting

Screen Readout





Shalem et al. Nat Rev Gen 2015

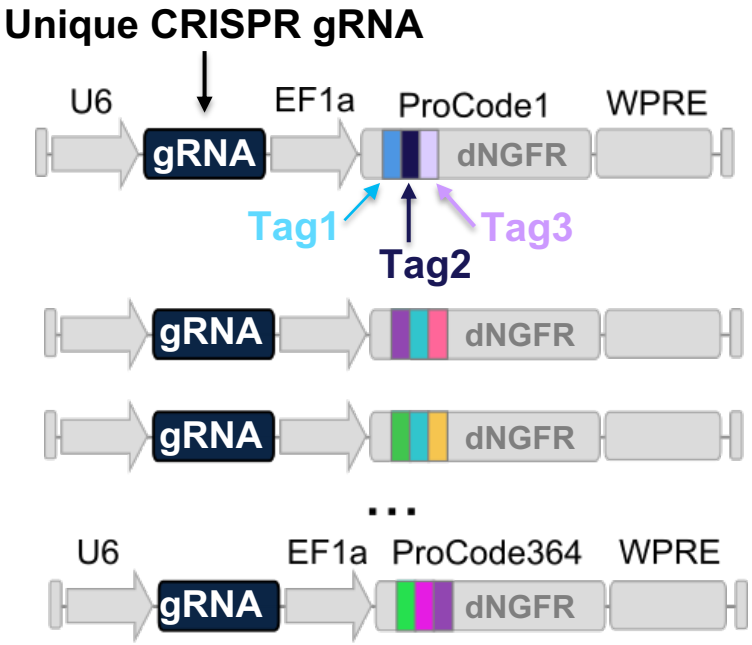
Functional Genomics has major limitations

- Mostly limited to screening for genes that impact cell fitness (i.e. cell proliferation or cell death)
- Phenotyping can only be inferred and is limited to single trait.
- Analysis is made on bulk populations (i.e. not single cell resolution)
- Requires substantial phenotype, and high penetrance, to detect
- In situ (spatial) analysis is not possible
- Limited to screening for genes with cell intrinsic functions

Question: Can we generate a barcoding system that permit phenotypic analysis and single cell resolution?

Hypothesis: A protein-based barcoding system would enable barcode detection by high resolution means, such as FACS, CyTOF and microscopy

Protein Barcodes (Pro-Codes) – a CRISPR barcoding system enabling high-dimensional phenotypic screen at a single cell resolution

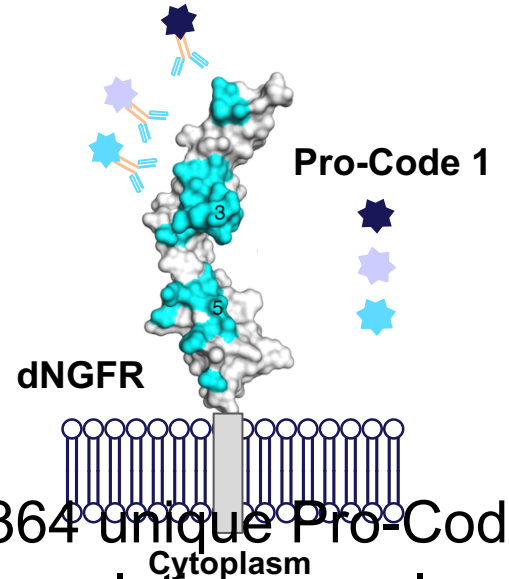


$$C(n,r) = \frac{n!}{r!\,(n-r)!}$$

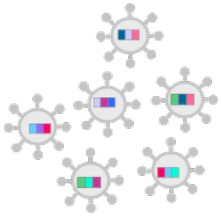
n (tags)
r (positions)
C (Pro-Codes)

$$\underline{C(14,3) = \mathbf{364}}$$

14 tags 3 spots = 364 Pro-Codes

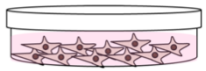


364 unique Pro-Code populations resolved



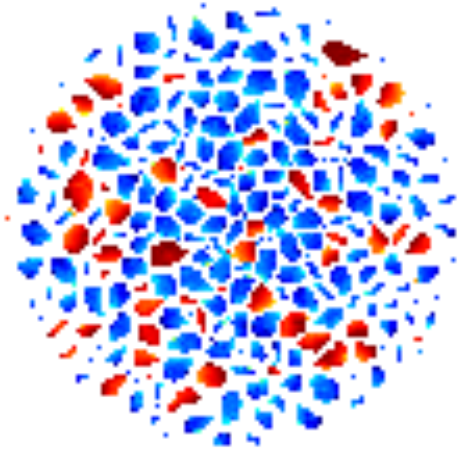
Pro-Code/ CRISPR library

Transduce (single copy)



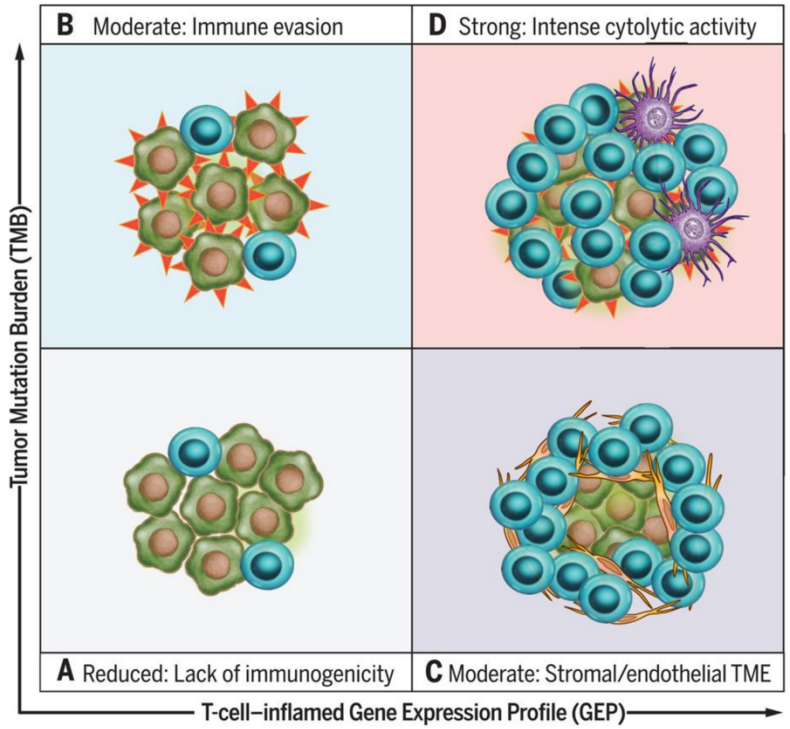
Stain for Pro-Codes (~10 Tags) + Phenotypic markers (10 – 25 proteins)

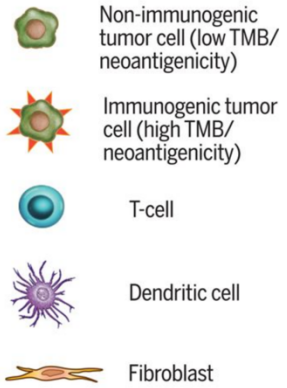
- FACS
- CyTOF



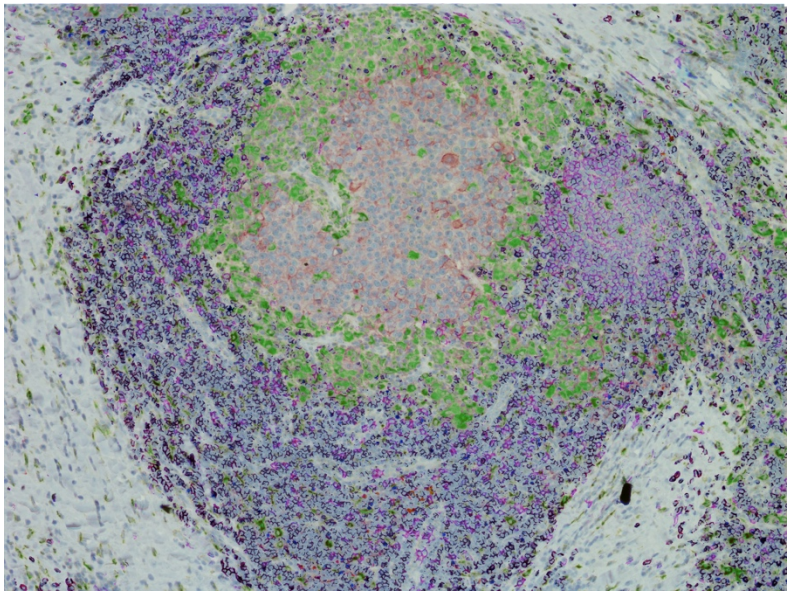
Wroblewska, Dhainaut et al. Cell 2018

Tumor immune composition is a major determinant of cancer outcome and response to immunotherapy





Cristescu et al, Science 2018



PD-L1 CD68 DC-LAMP CD20 CD3 FoxP3

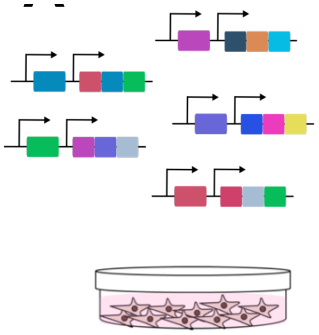
Remark et al, Science Immunol 2016

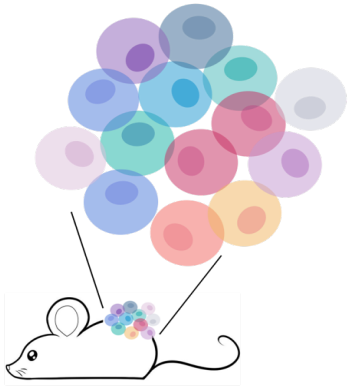
How can we identify the genetic determinants of cancer immunity in vivo at high scale?

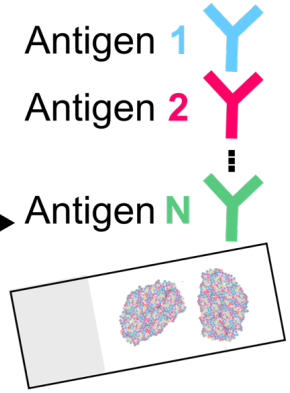
- CRISPR screens are limited to cell autonomous effects.
- The function of whole classes of genes (e.g. cytokines, chemokines,…) can therefore not be properly assessed using current approaches of CRISPR genomics.
- We need CRISPR genomics methods that retain the spatial information.

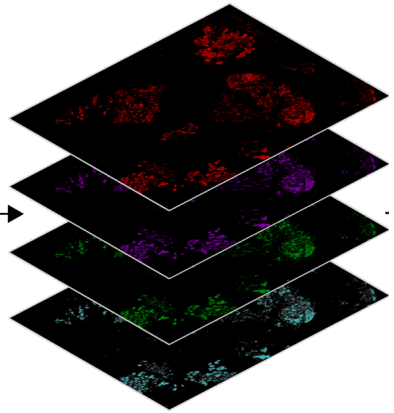
Multiplex imaging of Pro-Codes to spatially map gene perturbations in situ

Pro-Code/CRISPR library



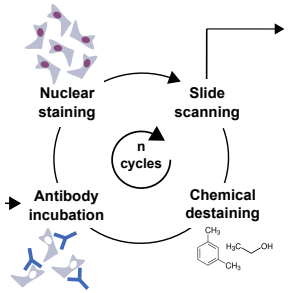


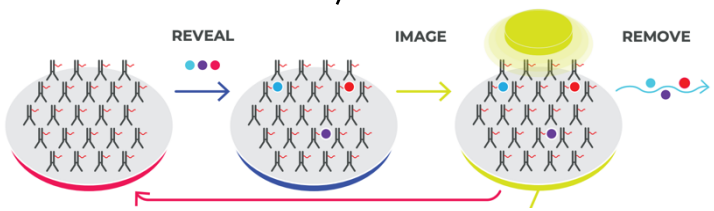


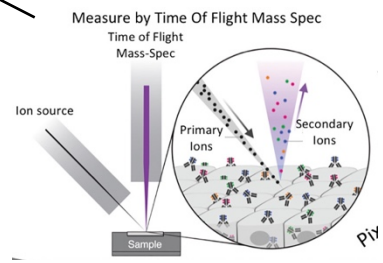


Multiplex Imaging

- MICSSS, CyCIF
- MIBI, Hyperion (aka tissue CyTOF)
- CODEX

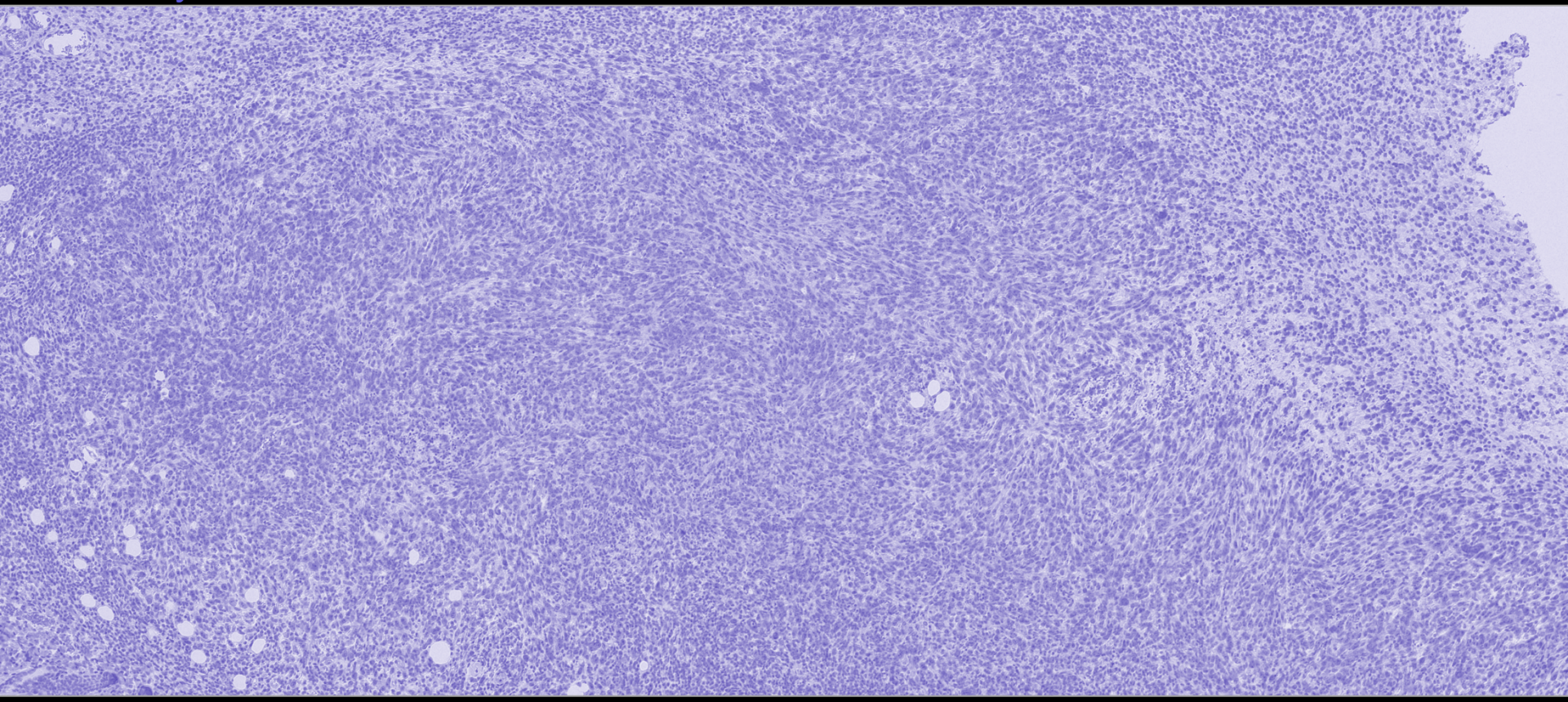


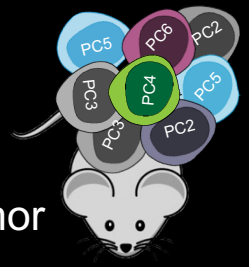




4T1 breast tumor marked with 85 different Pro-Codes

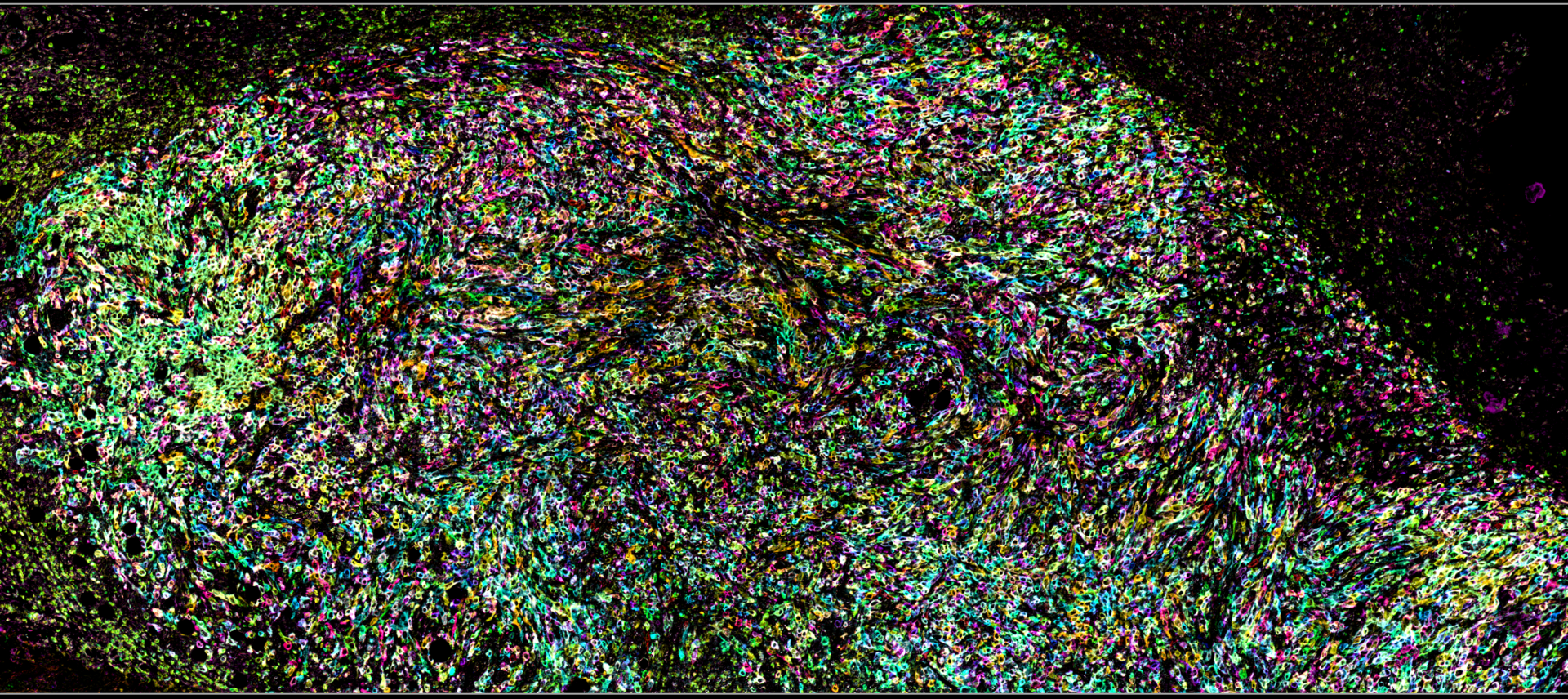
Hematoxylin





4T1 breast tumor

Spatial clonal mapping provides evidence of high cancer cell mobility within 4T1 breast tumors

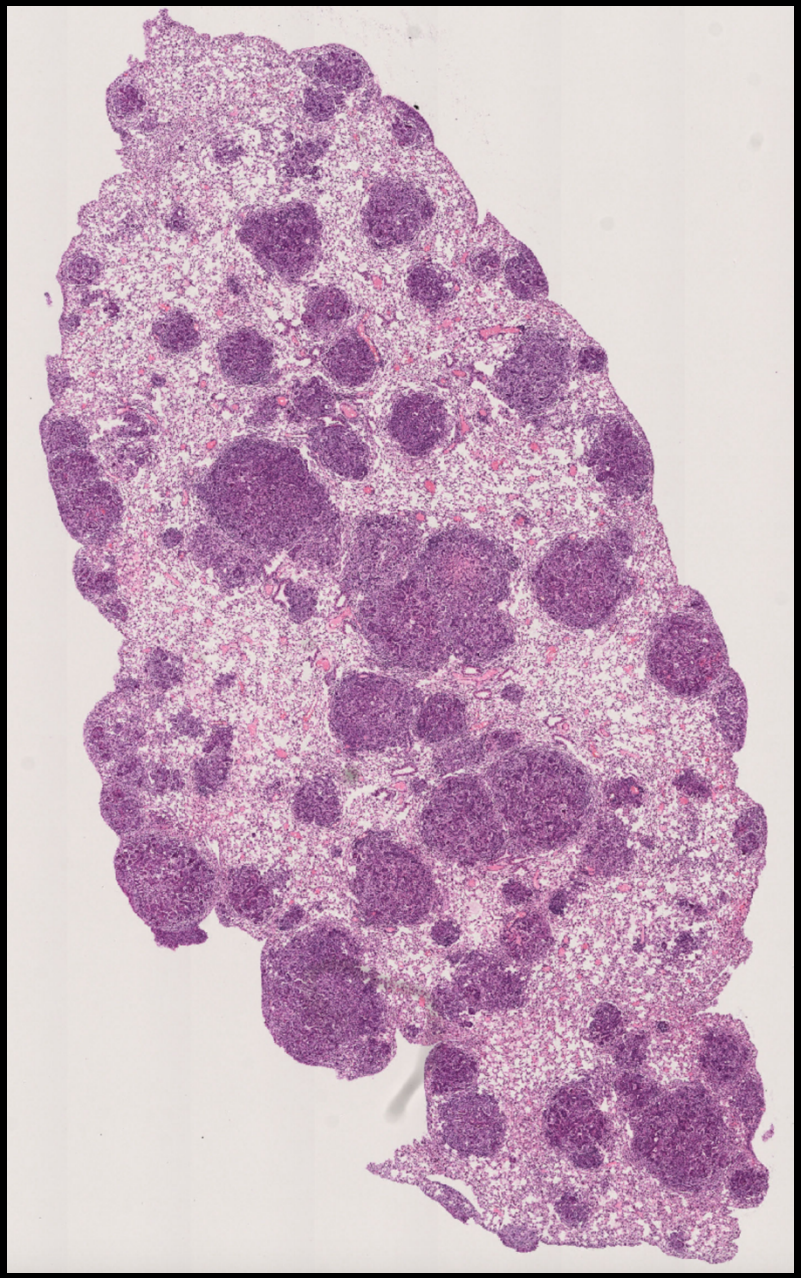


Pro-Code Tag 1 Pro-Code Tag 2 Pro-Code Tag 3

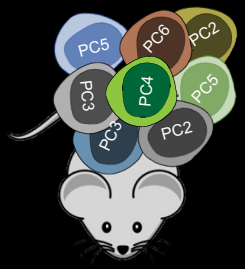
Pro-Code Tag 4 Pro-Code Tag 5 Pro-Code Tag 6

6 of 9 tags
(85 Pro-Code
populations)

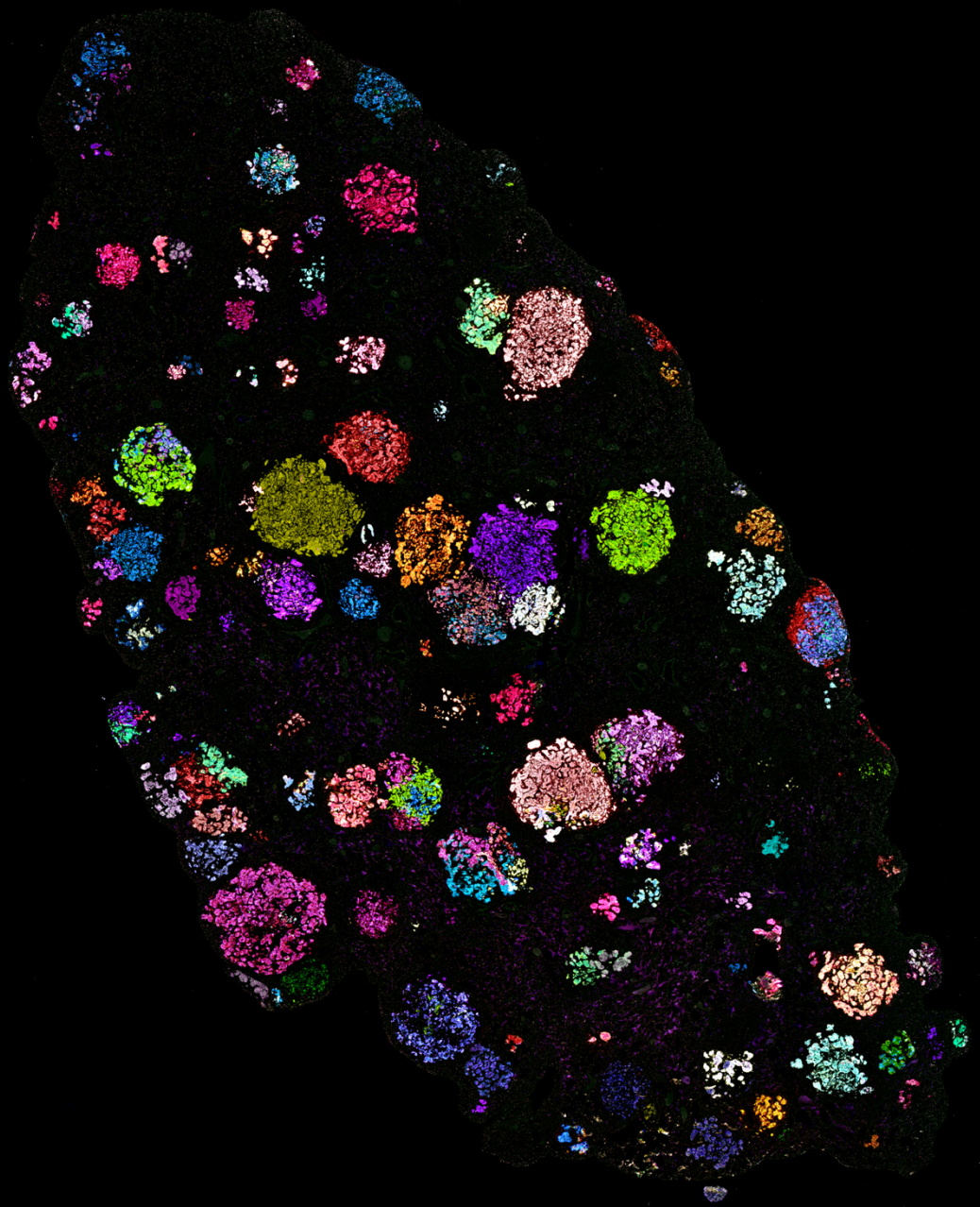
Spatial mapping of Pro-Code / KP lung tumors lesions



$Kras^{G12D}$, $p53^{null}$
KP lung tumor
w/ 85 Pro-Codes



Spatial mapping of Pro-Code / KP lung tumors lesions



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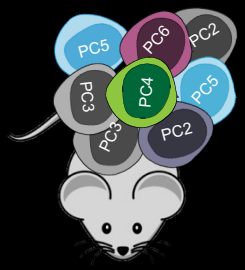
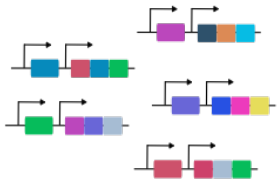
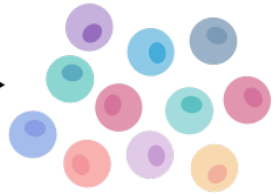
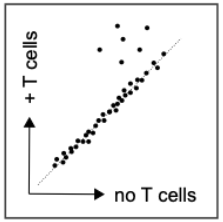


Pro-Code/CRISPR Genomics

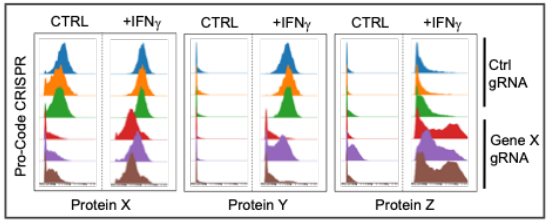




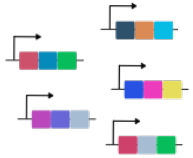
CyTOF analysis

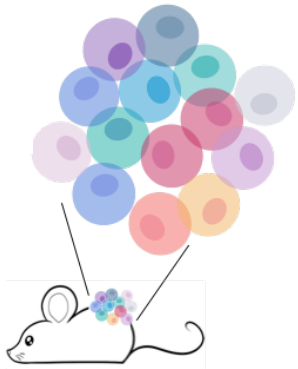


Sensitivity to immunoediting

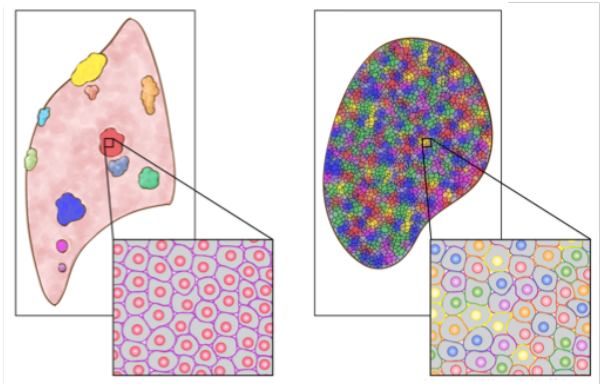


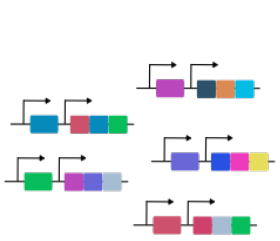
Cell phenotyping

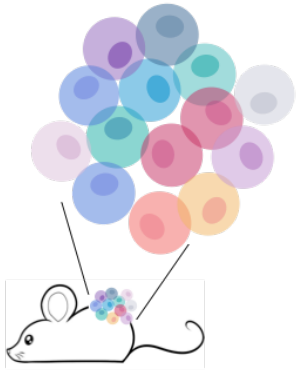


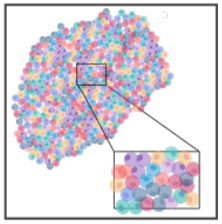


Spatial mapping

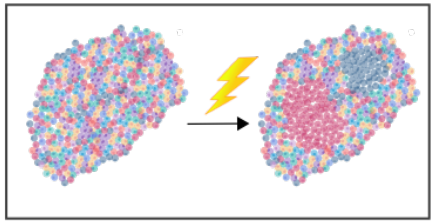




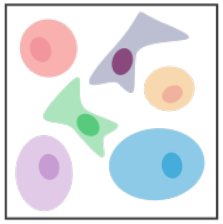




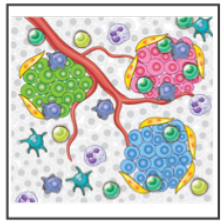
Spatial Mapping



Selection



Cell Morphology & Cell Phenotyping



Cell-cell interactions

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